Supplementary information

MODULATION OF FUNCTIONALLY SIGNIFICANT CONFORMATIONAL EQUILIBRIA IN ADENYLATE KINASE BY HIGH CONCENTRATIONS OF TRIMETHYLAMINE OXIDE ATTRIBUTED TO VOLUME EXCLUSION

Suresh babu Nagarajan, Dan Amir, Asaf Grupi, David P Goldenberg, Allen P Minton, and Elisha Haas

Table SI-1

Apparent kinetic constants of wild-type adenylate kinase, mutant adenylate kinases and their labeled derivatives[a].

	ATP[b] as variable substrate		AMP[c] as variable substrate	
Enzyme	K_m (ATP) (μM)	V_{max} (ATP) (IU/mg)	K_m (AMP) (μM)	V_{max} (AMP) (IU/mg)
Wild-type	96 ± 2	1046 ± 6	37 ± 1	1268 ± 34
C77S	94 ± 2	938 ± 6	36 ± 2	1268 ± 34
C77S,A203C	101 ± 3	978 ± 9	36 ± 2	1254 ± 24
C77S,A203C-**Cca**	162 ± 2	1140 ± 5	58 ± 2	1444 ± 20
C77S,V142W	96 ± 3	889 ± 6	36 ± 2	1210 ± 21
C77S,V142W,A203C	98 ± 3	1070 ± 9	38 ± 2	1405 ± 22
C77S,V142W,A203C-**Cca**	173 ± 3	964 ± 5	54 ± 4	1170 ± 40

Table SI 2 : Spectroscopic properties of labeled mutants [a]

mutant	Absorption		Fluorescence	
	λ_{max}^{abs} [b] (nm)	ε_{max} [b] ($M^{-1}cm^{-1}$)	λ_{max}^{em} (nm)	r[c]
W[142]-AK	278	18410	353	0.051±0.004
Cca[203]-AK	331	16500	451	0.174±0.008

[a] Measurements were carried out in 100 mM Tris -HCl pH 7.5 (25°C). [b] Data for the longest wavelength absorption band. [c]steady state anisotropy in the apoAK (similar values were obtained for the holoAK.

Table SI3. Parameters of the interdomain distance distribution of the "reverse mutant" labeled by a Trp residue at site 203 and a Cca probe attached to a Cys residue at site 142.

	Holo with AP4A	Holo with AP5A	apo
Mean of the distribution (Å)	24.6	24.2	30.8
FWHM (Å)	11.2	10.8	10.4
Transfer efficiency (%)	64	61	25
χ^2	1.29	1.32	1.19

Table SI-4 – Covolumes of the four conformational states of AK and a hard spherical cosolute. r is given in units of Å and covolumes in units of $Å^3$.

r	AK-open [1]	AK-AMP [4]	AK-ATP [3]	AK-closed [2]
2	4.79E+04	4.676E+04	4.722E+04	4.690E+04
3	5.93E+04	5.786E+04	5.811E+04	5.665E+04
4	7.11E+04	6.907E+04	6.921E+04	6.663E+04
5	8.35E+04	8.075E+04	8.067E+04	7.706E+04
10	1.57E+05	1.495E+05	1.463E+05	1.390E+05
20	3.70E+05	3.554E+05	3.439E+05	3.297E+05
30	6.98E+05	6.734E+05	6.544E+05	6.273E+05
40	1.17E+06	1.132E+06	1.103E+06	1.062E+06
50	1.80E+06	1.757E+06	1.710E+06	1.660E+06

Figure SI-1. Separation of AK derivatives by anion exchange chromatography on a Mono Q column (FPLC). Elution profiles of W_{142}-AK (A), W_{142}-/Cca_{203}AK (B) and **Cca**$_{203}$-AK (C).

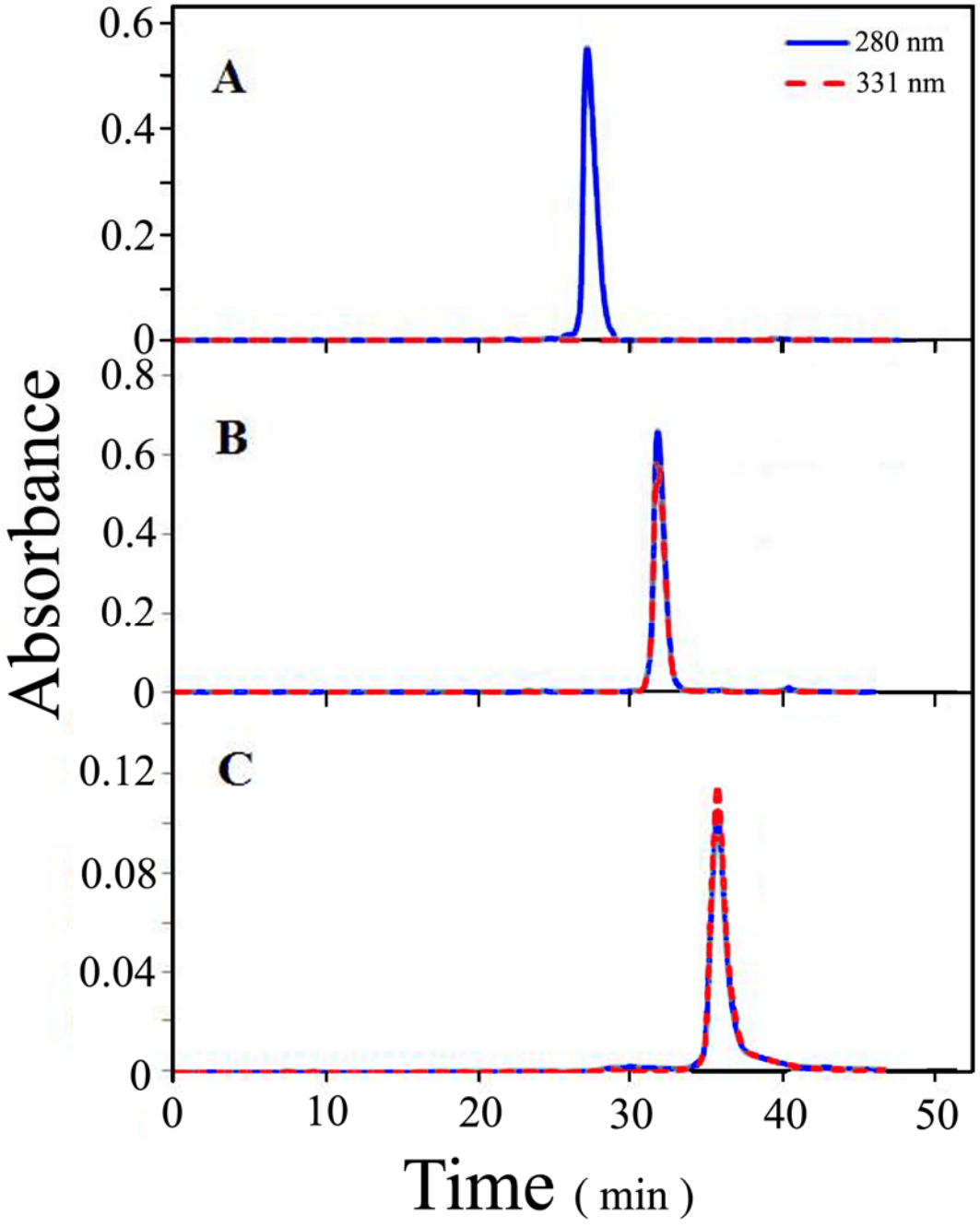


Figure SI-2 far UV CD of the labeled mutants and the wt AK.

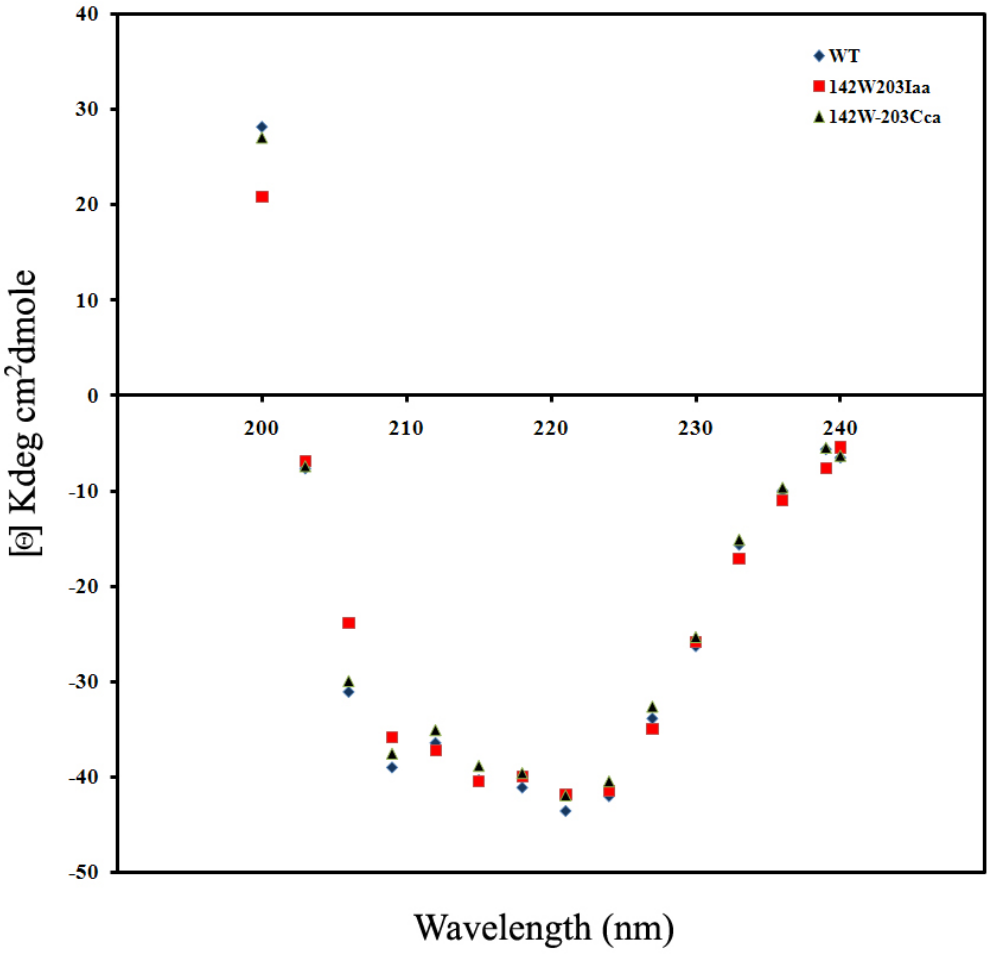


Figure SI-3 the interdomain distance obtained for the "reverse mutant" a mutant labeled by Trp residue at residue 203 and the acceptor, Cca, attached to a Cys residue 142 in the absence of TMAO. The results are the same as obtained for the mutant labeled with a Trp residue at site 142 and the acceptor at residue 203. the results are given in Table SI 6.

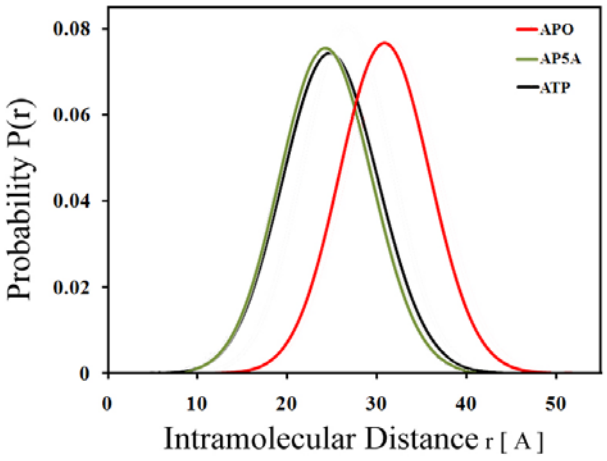


Figure SI-4. Calculated changes of covolume associated with conversion of conformational state [1] to conformational states [2] – [4], plotted as a function of *r*, the radius of a hard spherical probe. Symbols: calculated from values given in Table S1. Curves: text equations [8] – [10].

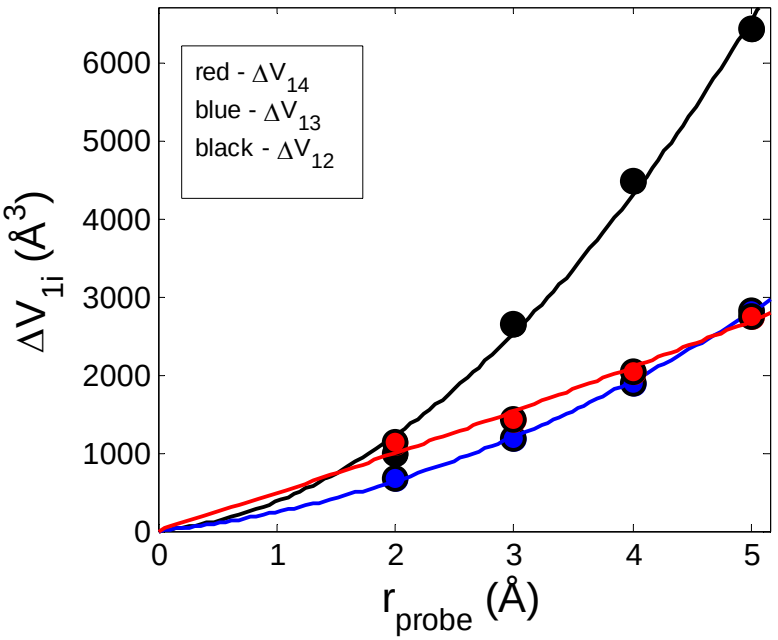
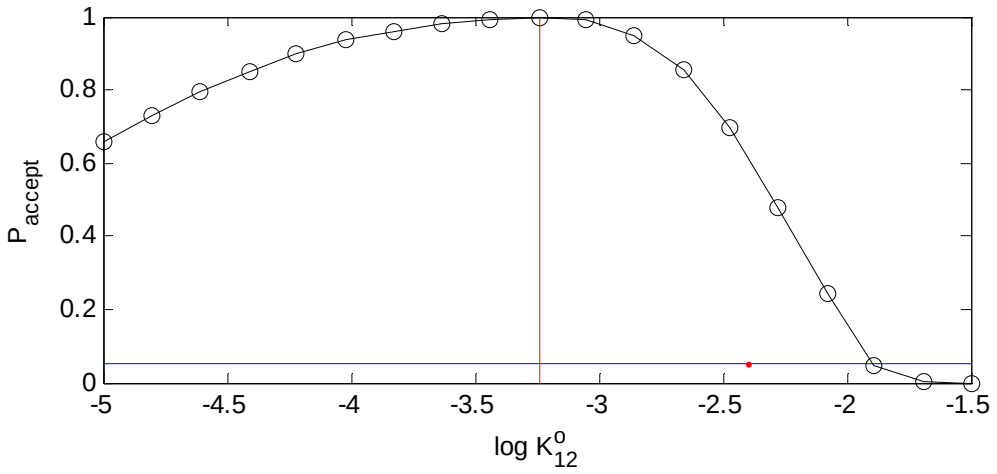
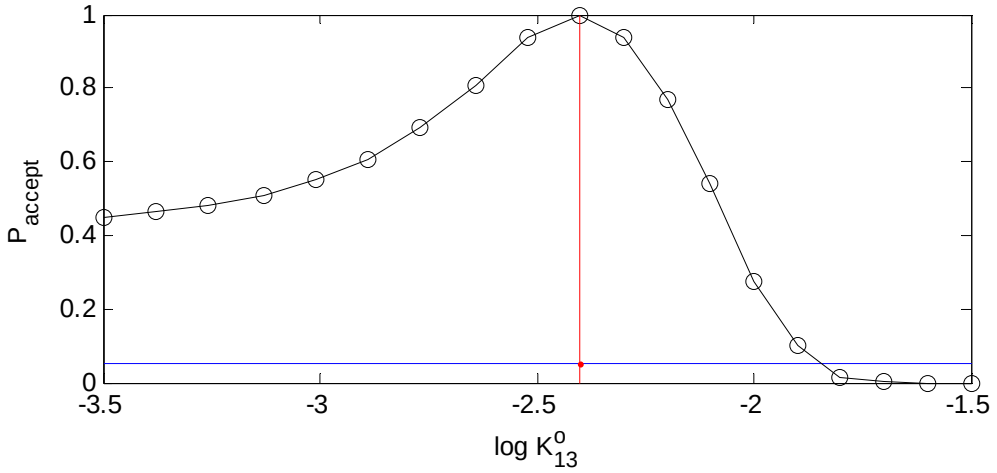
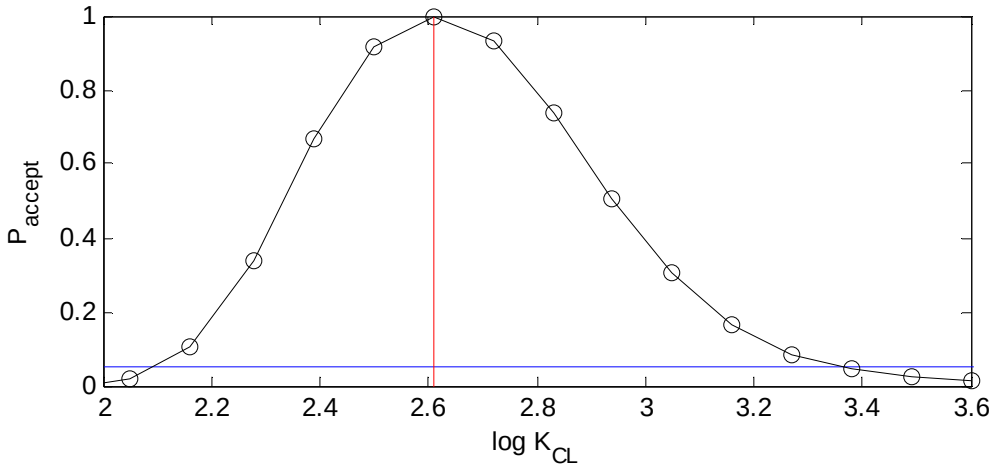
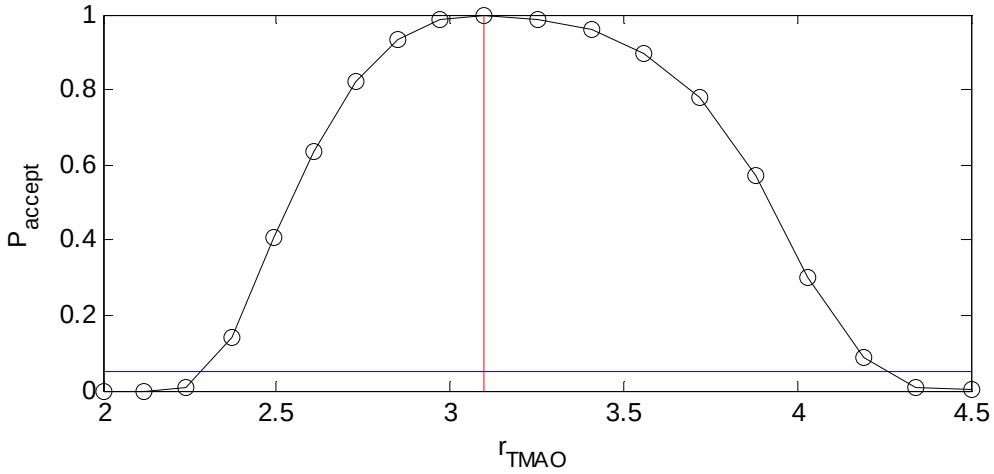


Figure SI-5 – Estimate of the uncertainty of best-fit parameters derived from least-squares modeling of four conformational state model to dependence of fraction of closed conformations upon concentrations of ATP and TMAO. Values of each parameter are constrained at values above and below the best-fit value, and the values of all other parameters allowed to vary to obtain a constrained best fit. The ratio of the variance (sum of squared residuals) of the constrained best fit to that of the unconstrained best fit is assumed to be distributed according to the Fisher F-statistic. P_{accept} is defined as the probability, calculated according to the Fisher F-test, that the ratio of variances calculated for a particular value of the selected parameter does not exceed the value of the F-statistic calculated for the appropriate number of mathematical degrees of freedom of numerator and denominator. According to the F-test, the probability that the true value of a given parameter lies between the lesser and greater values of that parameter with a value of P_{accept} is 1 - P_{accept} .






1
0.8
0.6
0.4
0.2
0
P accept
2
2.2
2.4
2.6
2.8
3
3.2
3.4
3.6
log K CL


1
0.8
0.6
0.4
0.2
0
P accept
2
2.5
3
3.5
4
4.5
r TMAO

Calculation of the size of a hard-spherical representation of TMAO

To determine the appropriate radius of an equivalent hard sphere representing TMAO, the covolumes of a TMAO model with a series of spheres of increasing radii were calculated. The model was constructed from the chemical formula of TMAO using the online version of the program CORINA (Molecular Networks, `http://www.molecular-networks.com/online_demos/corina_demo`. As in the covolume calculations with proteins described in the text, only non-hydrogen TMAO atoms were included, and the Chothia group radii were used. The radii of the spheres used for these calculations ranged from 0.5 to 20 Å. The resulting covolumes were then plotted as a function of the sphere radius r_s, (Figure SI 6) and fit by the method of least squares to the expression for the covolume of two spheres:

$$V = \frac{4\pi}{3}(r_s + r_m)^3 \qquad (1)$$

where r_m is the radius of a sphere representing the TMAO molecule. An excellent fit was obtained with $r_m = 2.74\text{Å}$.

Figure SI 6. Points: calculated covolume of a molecular model of TMAO with hard spheres of varying radii. Curve: calculated covolume of a hard sphere of radius 2.74 Å with a hard sphere of arbitrary radius.